

Inhibitory effects of organic acid salts on growth of *Clostridium perfringens* from spore inocula during chilling of marinated ground turkey breast[☆]

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Abstract

Inhibition of *Clostridium perfringens* germination and outgrowth by salts of organic acids such as sodium lactate, sodium acetate, buffered sodium citrate and buffered sodium citrate supplemented with sodium diacetate was evaluated during continuous chilling of ground turkey. Turkey breast meat was injected with a brine-containing NaCl, potato starch and potassium tetra pyrophosphate to yield final in-product concentrations of 0.85%, 0.25% and 0.20%, respectively. The meat was ground, mixed with either sodium lactate (1%, 2%, 3% or 4%), sodium acetate (1% or 2%), buffered sodium citrate (Ional[™], 1%) or buffered sodium citrate supplemented with sodium diacetate (Ional Plus[™], 1%), in addition to a control that did not contain added antimicrobials. Each product was mixed with a three-strain *C. perfringens* spore cocktail to obtain final spore concentrations of ca. 2.8 log₁₀ spores/g. Inoculated products (10 g) were packaged into cook-in-bags (2 × 3 in.), vacuum sealed, cooked at 60 °C for 1 h, and subsequently chilled from 54.4 to 7.2 °C in 15, 18 and 21 h following exponential chilling rates. Products were sampled immediately after cooking and then after chilling. Chilling of cooked turkey following 15, 18 and 21 h chill rates resulted in germination and outgrowth of *C. perfringens* spores to 6.6, 7.58 and 7.95 log₁₀ CFU/g populations, respectively, from initial spore populations of ca. 2.80 log₁₀ CFU/g. Incorporation of sodium lactate (1%), sodium acetate (1%), Ional or Ional Plus (1%) substantially inhibited germination and outgrowth of *C. perfringens* spores compared to controls. Final *C. perfringens* total populations of 3.12, 3.10, 2.38 and 2.92 log₁₀ CFU/g, respectively, were observed following a 15-h exponential chill rate. Similar inhibitory effects were observed for 18 and 21 chill rates with the antimicrobials at 1% concentrations. While sodium lactate and sodium acetate concentrations of 1% were sufficient to control *C. perfringens* germination and outgrowth (<1.0 log₁₀ CFU/g growth) following 15 h chill rates, higher concentrations were required for 18 and 21 h chill rates. Ional at 1% concentration was effective in inhibiting germination and outgrowth to <1.0 log₁₀ CFU/g of *C. perfringens* for all three chill rates (15, 18 and 21 h) tested. Use of

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sodium salts of organic acids in formulation of ready-to-eat meat products can reduce the risk of *C. perfringens* spore germination and outgrowth during chilling.

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1. Introduction

Clostridium perfringens is an important foodborne pathogen and is estimated to cause 248,000 cases of foodborne illness in the United States annually (Mead et al., 1999). Several processed meat products, such as roast beef, turkey and meat-containing Mexican foods have been implicated in *C. perfringens* outbreaks (Bryan, 1978). The spores of *C. perfringens* are widely distributed in soil and water and often contaminate raw meat and poultry during slaughter operations. The U. S. Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) reported *C. perfringens* prevalence rates of 29.2% and 42.9% for turkey (357 positive out of 1221 samples; August 1996 through July 1997) and broiler (557 positive out of 1297 samples; July 1994 through June 1995) rinse fluids, respectively. Mean *C. perfringens* populations of 1.60 log₁₀ CFU/g were reported for ground turkey in a nationwide baseline study conducted in 1995 (USDA-FSIS, 1995).

C. perfringens outbreaks occur primarily as a result of consumption of foods that are improperly handled after cooking (Blankenship et al., 1988). Decimal reduction times ($D_{58\text{ }^{\circ}\text{C}}$ values) of 1.15–1.60 min for 10 strains of *C. perfringens* (vegetative cells) were reported in a model beef gravy system (Juneja et al., 2001). Accordingly, thermal processing schedules designed to achieve a 6.5-D reduction in *Salmonella* spp. should also be adequate to destroy vegetative cells of *C. perfringens*. However, spores of this organism, if present in raw materials used in preparation of meat products, can survive the traditional heat processing schedules employed by the meat industry. The heat-activated surviving spores pose a potential public health hazard due to the potential to germinate, outgrow and multiply during subsequent chilling operations, especially when proper chill rates are not followed or when the products are not properly refrigerated or are temperature abused.

The USDA-FSIS compliance guidelines (USDA-FSIS, 1999, 2001) for chilling of thermally processed meat and poultry products state that the products should be chilled from 54.4 to 26.7 °C within 1.5 h, and further to 4.4 °C within an additional 5 h (Option I). However, other options are available, such as chilling the product from 48 to 12.7 °C in 6 h with continued cooling to 4.4 °C, with the stipulation that the product not be shipped until the temperature reaches 4.4 °C (Option II). The USDA-FSIS stated that the latter guideline yields a significantly smaller margin of safety than the former, especially when a non-intact product is cooled using this guideline. Further, the option II guideline indicates that product cooling should be rapid between 48 and 26.7 °C within 1 h, and that the product cooling should be closely monitored. For cooked, cured meat products, with a minimum of 100 ppm sodium nitrite (ingoing), the product can be cooled from 54.4 to 26.7 °C in 5 h and further to 4.4 °C within an additional 10 h (Option III). If the cooling guidelines can not be achieved, the USDA-FSIS guidelines specify that computer modeling using the USDA-Pathogen Modeling Program and/or product sampling can be used to evaluate the severity and microbiological risk of the process deviation. However, these guidelines do not take into consideration the antimicrobial efficacies of the organic acid salts that are increasingly being used by meat processors, either as flavor enhancers or microbial inhibitors.

Cooking and cooling of solid food products results in an exponential increase or decrease in temperature at the core of the product when exposed to a cooking or a cooling regime (Juneja et al., 1994). The authors reported minimal *C. perfringens* growth (<1.0 log₁₀ CFU/g) from heat shocked spores in cooked beef during exponential cooling from 54.4 to 7.2 °C at rates varying from 6 to 15 h. However, longer chill rates (18 h) resulted in >1.0 log₁₀ growth in *C. perfringens* from spore inocula.

The USDA-FSIS performance standard for germination and outgrowth of sporeforming bacteria specifies that germination and outgrowth should be $< 1.0 \log_{10}$ CFU/g, and that *C. perfringens* can be used in a microbial challenge study to demonstrate that the cooling performance standard is met for both *C. perfringens* and *C. botulinum* (USDA-FSIS, 1999, 2001).

Salts of organic acids such as sodium or potassium lactate and sodium diacetate are extensively used in meat and poultry products to enhance the microbiological safety of these products by controlling *Listeria monocytogenes* and other foodborne spoilage organisms (Harmayani et al. 1993; Schlyter et al. 1993). Buffered sodium citrate is used to enhance the flavor of these products (USDA-FSIS, 1996). While sodium and potassium lactate use levels up to 4.8% are permitted as microbial inhibitors, use of sodium acetate and diacetate as flavor enhancers should be limited to less than 0.25% by weight of total formulation (USDA-FSIS, 2000). Thippareddi et al. (2003) showed that the germination and outgrowth of *C. perfringens* could be controlled by use of buffered sodium citrate in the formulation during chilling of cooked roast beef and injected pork products.

The present study evaluated use of sodium lactate, sodium acetate and buffered sodium citrate (alone and in combination with sodium diacetate) to control or inhibit germination and outgrowth of *C. perfringens* from spores in processed turkey products during extended chill situations.

2. Materials and methods

2.1. *C. perfringens* cultures and spore production

C. perfringens strains NCTC 8238 (Hobbs serotype 2), NCTC 8239 (Hobbs serotype 3) and NCTC 10240 (Hobbs serotype 13) were used in this study. *C. perfringens* spore preparation and origin and sources of the strains are described by Juneja et al. (1993). Briefly, a spore crop of each strain was prepared separately, washed twice, re-suspended in sterile distilled water and stored at 4 °C for up to 2 months. Approximately equal numbers of spores of each of the three *C. perfringens* strains were mixed to prepare a spore cocktail, immediately prior to experimentation,

by mixing ca. 1 ml from each suspension. This spore mixture (0.75 ml) was then mixed with the injected turkey breast meat (250 g of each product) for 1 min in a KitchenAid mixer (K5SSWH model; KitchenAid, Troy, OH), vacuum packaged (Model No. C-500, Multivac, Kansas City, MO; Cryovac, L340 bag, water vapor transmission rate: 10 g/1 m²/24 h at 37.8 °C, 100% RH; oxygen transmission rate: 3000 cm³/1 m²/24 h at 23 °C, 1 atm.) and frozen (–80 °C) until used.

2.2. Preparation of the meat and inoculation

Procedures described by Thippareddi et al. (2003) for preparation of meat products were followed. Briefly, turkey breast meat (boneless) was obtained from a retail store (Athens, GA), and injected with minimal final levels of salt (NaCl, 0.85%), potato starch (0.25%) and potassium tetra pyrophosphate (0.2%). The products were vacuum packaged separately and shipped overnight with ice packs to the Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA, and were stored under refrigeration until use. The products were diced and ground through a 3.17-mm plate (Model 4822; Hobart, Troy, OH) to subsequently aid in uniform distribution of the antimicrobial ingredients and *C. perfringens* spores. Separate packages containing 250 g of meat were prepared, vacuum packaged and stored frozen. The products were thawed, mixed with sodium lactate (1%, 2%, 3% and 4%; Sigma, St. Louis, MO), sodium acetate (1% and 2%; Sigma), buffered sodium citrate (BSC; Ional™, 1%) (WTI, Kingston, NY) or BSC supplemented with sodium diacetate (Ional Plus™, BSC with 8.0% sodium diacetate, 1%) (WTI) for 1 min in a mixer (KitchenAid) and subsequently mixed with the spore cocktail for 1 min to yield ca. 2.8 log₁₀ spores/g. The product (injected turkey breast meat; 10 g) was distributed into sterile filter stomacher bags (SFB-510, Spiral Biotech, Boston, MA), and vacuum sealed at 12 mbar vacuum using a Multivac (Model A300/16, Multivac) packaging machine.

2.3. Treatments

Seven treatments (sodium lactate: 1%, 2%, 3% and 4%; sodium acetate 1% and 2%; BSC (Ional™) or BSC with sodium diacetate (Ional Plus™) at 1%),

along with an untreated control, to inhibit germination and outgrowth of *C. perfringens* spores were evaluated.

2.4. Heat shock and cooling procedures

The bags with inoculated products were sandwiched between stainless steel wire racks as described by Thippareddi et al. (2003), submerged completely in a circulating water bath set at 60 °C (Exacal, Model RTE-221, NESLAB Instruments, Newington, NH). As such, the products/spores were cooked/heat shocked for 1 h, removed, chilled immediately in an ice water bath and plated as described below. A second set of racks containing the product for each treatment was heat shocked as described and transferred to a programmable water bath (Exacal, Model RTE-221, NESLAB Instruments) set at 54.5 °C, allowed to equilibrate at this temperature for 10 min and chilled at an exponential rate from 54.5 to 7.2 °C according to the target chilling times (Table 1).

Table 1

Target product temperatures during cooling of injected turkey breast meat containing *C. perfringens* spores from 54.4 to 7.2 °C

Elapsed time (h)	Cooling time from 54.4 to 7.2 °C		
	15 h (°C)	18 h (°C)	21 h (°C)
1	47.6	48.7	49.5
2	41.6	43.5	44.9
3	36.4	38.9	40.8
4	31.8	34.8	37.1
5	27.8	31.1	33.7
6	24.3	27.8	30.6
7	21.2	24.8	27.8
8	18.5	22.2	25.2
9	16.2	19.8	22.9
10	14.1	17.7	20.8
11	12.4	15.8	18.9
12	10.8	14.1	17.1
13	9.4	12.6	15.6
14	8.2	11.3	14.1
15	7.2	10.1	12.8
16		9.0	11.7
17		8.1	10.6
18		7.2	9.6
19			8.7
20			7.9
21			7.2

2.5. Enumeration procedure

Immediately after cooking and/or chilling, sterile peptone water (PW, 0.1%; 10 ml) was added to the meat samples, and contents were stomached for 2 min (Interscience, St. Nom, France). The samples were serially diluted in PW and plated on tryptose sulfite cycloserine (TSC; Difco, Detroit, MI) agar by pour or spiral-plating methods as described previously (Juneja and Marmer, 1998). The plates were overlaid with an additional 10 ml of TSC, incubated at 37 °C for 18–24 h in a Bactron anaerobic chamber (Bactron IV, Sheldon Laboratories, Cornelius, OR) before counting *C. perfringens* colony forming units.

2.6. Statistical analyses

Three independent trials, as defined by a new batch of meat, were performed for each of the exponential chilling rates (15, 18 and 21 h). Data were analyzed by analysis of variance using the General Linear Model procedure of the Statistical Analysis System (SAS Institute, Cary, NC, 2000; Release 8.01). Bonferroni LSD method was used to separate means of the *C. perfringens* populations (log₁₀ CFU/g) (Miller, 1981).

3. Results and discussion

The programmed temperature profiles of the products for the 15, 18 and 21 h exponential chill rates are shown in Table 1. These temperature profiles represent extended chilling rates in view of the USDA-FSIS or the FDA stabilization requirements for chilling of uncured, cooked meat and poultry products (USDA-FSIS, 1999; FDA, 2001). The mean pH of injected turkey breast meat was 5.72–6.06 (results not shown) and corresponds to the normal pH of these products reported in the literature (Faustman, 1994). Compared to the pH of roast beef (5.62) and injected pork (6.11), the pH of the injected turkey was intermediate (pH 5.94) in the untreated control (Thippareddi et al., 2003). These differences are natural muscle pH differences and were not affected by the minimal phosphate levels used for injection (Faustman, 1994; Lyon and Lyon, 2001). Heat treatment and chilling did not alter the pH of

any of the formulated products (results not shown). However, differences in pH were observed within the treatments. Addition of Ional and Ional Plus reduced the pH of the product to pH 5.77 and 5.72, respectively ($p \leq 0.05$), while addition of sodium lactate and sodium acetate at 1% concentrations appreciably increased ($p \leq 0.05$) the pH of the product to pH 6.01 and 6.06, respectively, compared to pH 5.96 of the untreated control (results not shown). Whether these minimal changes, although statistically significant, would affect germination and outgrowth of *C. perfringens* spores is debatable.

Chilling of injected turkey breast meat from 54.4 to 4.4 °C resulted in germination and outgrowth of *C. perfringens* spores from initial populations of 2.77, 2.92 and 2.88 log₁₀ CFU/g to 6.60, 7.58 and 7.95 log₁₀ CFU/g following 15, 18 and 21 h exponential chill rates, respectively (Figs. 1, 2 and 3). These germination and outgrowth results exceed the USDA-FSIS stabilization performance standards for control of *C. perfringens*. Steele and Wright (2001) reported that chilling cooked, ready-to-eat turkey breast roasts from 48.9 (120) to 12.8 °C (55 °F) within 8.9 h resulted in <1.0 log₁₀ CFU/g growth in *C. perfringens* from spore inocula. The chill times (15, 18 and 21 h between 54.4 and 4.4 °C) followed in our study for the temperature range 48.9–12.8 °C correspond to 10, 12 and 14 h reported by Steele and

Wright (2001), and are beyond the safe chill rates proposed (8.9 h). Our results are in agreement with those reported by Steele and Wright (2001) in which the authors used whole turkey breast roasts (4 kg each). In the present study, we have validated the use of small flexible polythene bags for conducting challenge study using *C. perfringens* spore inoculated product. The method used in the present study has the advantage of ease of use, with very small product amounts (10 g) and can be assumed to be at the center of the large turkey breast, considered to be the cold-spot (worst-case scenario). Further, the use of a water bath to generate the desired product temperature profiles is highly reproducible and can be used in future validation studies.

Use of sodium lactate, acetate, Ional or Ional Plus inhibited germination and outgrowth of *C. perfringens* spores when a 15-h exponential chill rate was followed (Fig. 1). Use of Ional (1%) and sodium lactate (>2%) or sodium acetate (2%) slightly reduced the *C. perfringens* populations following a 15-h chill rate, while Ional Plus (1%), sodium acetate (1%) and sodium lactate (1%) resulted in minimal growth of *C. perfringens* (<0.32 log₁₀ CFU/g). Similar reductions were observed in a previous study (Thippareddi et al., 2003) using roast beef and injected pork formulations, wherein the populations of *C. perfringens* after exponential chilling were lower compared

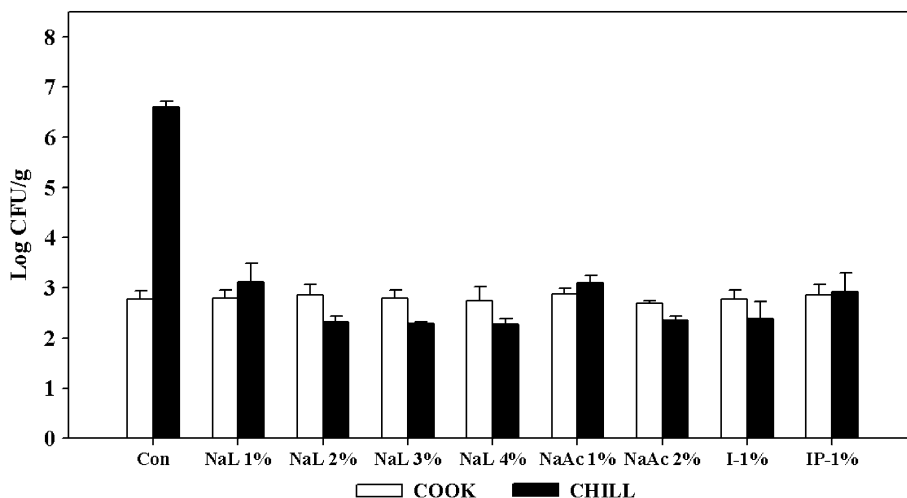


Fig. 1. Mean log CFU/g populations of *C. perfringens* in injected turkey breast immediately after heat shock (□: 60 °C for 1 h) and following cooling (■) from 54.4 to 7.2 °C exponentially in 15 h (I: Ional™; IP: Ional Plus™; NaL: sodium lactate; NaA: sodium acetate, 1% concentration of I or IP; 1%, 2%, 3% and 4% of NaL and 1% and 2% of NaA; Con: control).

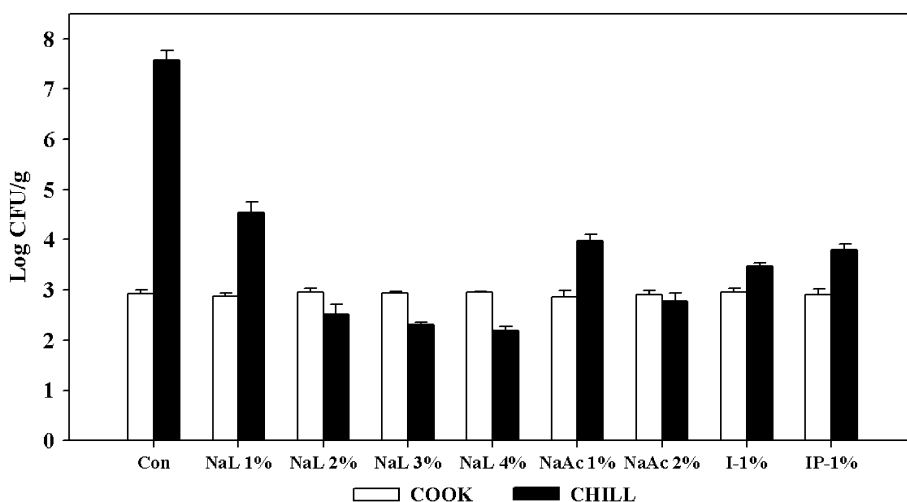


Fig. 2. Mean log CFU/g populations of *C. perfringens* in injected turkey breast immediately after heat shock (□: 60 °C for 1 h), and following cooling (■) from 54.4 to 7.2 °C exponentially in 18 h (I: Ional™; IP: Ional Plus™; NaL: sodium lactate; NaA: sodium acetate; 1% concentration of I or IP; 1%, 2%, 3% and 4% of NaL and 1% and 2% of NaA; Con: control).

to the populations after heat shock and/or before the start of chilling. Whether this is due to inactivation of germinated spores or the vegetative cells resulting from complete germination of a proportion of the spore population can not be determined, as our enumeration procedures evaluated only the total *C. perfringens* populations, and not populations of spores vs. vegetative cells.

Chilling of inoculated injected turkey breast meat from 54.4 to 4.4 °C in 18 h resulted in a 4.66 log₁₀ CFU/g increase in *C. perfringens* populations in the untreated control (Fig. 3). Incorporation of Ional and Ional Plus (1%) resulted in <1.0 log₁₀ CFU/g (0.51 and 0.88 log₁₀ CFU/g, respectively) increase in *C. perfringens* populations, while sodium lactate and sodium acetate (1%) resulted in >1.0 log₁₀ CFU/g

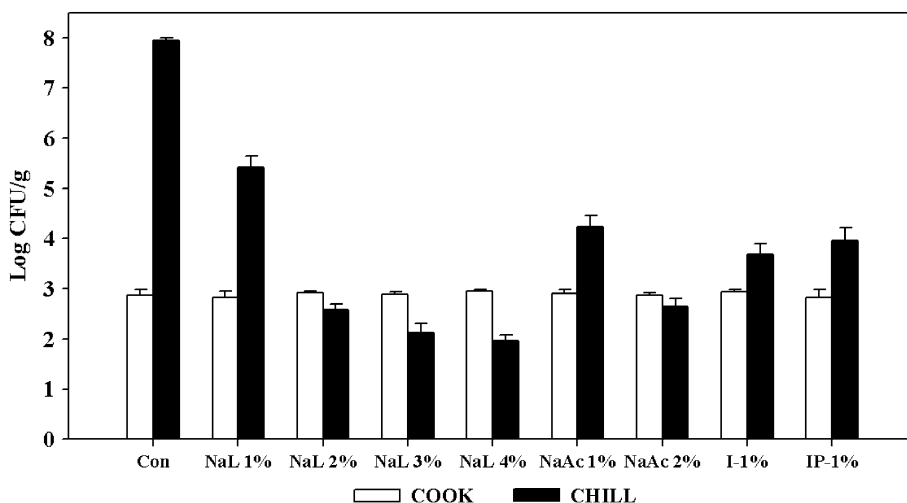


Fig. 3. Mean log CFU/g populations of *C. perfringens* in injected turkey breast immediately after heat shock (□: 60 °C for 1 h), and following cooling (■) from 54.4 to 7.2 °C exponentially in 21 h (I: Ional™; IP: Ional Plus™; NaL: sodium lactate; NaA: sodium acetate; 1% concentration of I or IP; 1%, 2%, 3% and 4% of NaL and 1% and 2% of NaA; Con: control).

increases (1.68 and 1.11 log₁₀ CFU/g, respectively). Incorporation of higher concentrations of sodium lactate ($\geq 2.0\%$) and sodium acetate (2.0%) resulted in decreases in total *C. perfringens* populations. A linear relationship between the increase in the concentration of sodium lactate and the decrease in *C. perfringens* populations was observed (0.44, 0.62 and 0.76 log₁₀ CFU/g decrease with 2%, 3% and 4% concentrations, respectively). Compared to roast beef and injected pork (Thippareddi et al., 2003), injected turkey allowed for greater germination and outgrowth of *C. perfringens* spores, with 4.30, 6.38 and 7.58 log₁₀ CFU/g after exponential chilling in 18 h from initial spore populations of 2.79, 2.68 and 2.92 log₁₀ CFU/g, respectively. Although the pH of injected turkey was intermediate compared to both roast beef and injected pork, the ability of *C. perfringens* spores to germinate and grow in turkey indicates that other factors may contribute to its control during chilling of meat products. Similar results were observed, with greater (>1.0 log₁₀ CFU/g) *C. perfringens* growth in injected turkey compared to roast beef and injected pork, for treatments with Ional and Ional Plus at 1% concentrations.

When injected turkey breast was chilled following a 21-h exponential chill rate, a 5.07 log₁₀ CFU/g increase in *C. perfringens* populations in untreated control was observed (Fig. 3). Addition of Ional, Ional Plus, sodium lactate or sodium acetate at 1% concentration resulted in increases of 0.74, 1.14, 2.58 and 1.33 log₁₀ CFU/g, respectively, subsequent to a 21-h exponential chilling from 54.4 to 4.4 °C. While decreases in total *C. perfringens* populations were observed following the same chill rate for roast beef and injected pork, increase of <1.0 log was observed when Ional and Ional Plus (1% concentration) were incorporated in injected pork (Thippareddi et al., 2003). Incorporation of sodium lactate at 2%, 3% and 4% concentrations resulted in 0.33, 0.76 and 0.98 log₁₀ CFU/g decreases in total *C. perfringens* populations, while addition of sodium acetate (2%) resulted in a 0.22 log₁₀ CFU/g decrease.

Juneja et al. (1994) reported germination and outgrowth of *C. perfringens* from 1.5 to 6.0 log₁₀ CFU/g during exponential cooling of autoclaved ground beef from 54.4 to 7.2 °C in 18 h. Minimal *C. perfringens* outgrowth was observed when ground beef was chilled within 15 h for the same temperature

decline, while 3.83 log₁₀ CFU/g growth was observed in the present study using injected turkey breast. These differences in the germination and outgrowth of *C. perfringens* from spore inocula could be due to pH differences in the meat substrate and/or other intrinsic characteristics of the product. Similar differences between meat substrates (beef, pork and turkey breast) were observed when the results obtained in this study are compared to those of Thippareddi et al. (2003). The procedures used by Thippareddi et al. (2003) for injection, marinate composition, meat injection rate and product chilling method were followed in the present study. It is obvious that *C. perfringens* germination and outgrowth rates differ with different substrates (meat types) and application of results from other substrates would misrepresent the potential hazard for its outgrowth in meat products.

Published literature on the inhibitory effects of chemical antimicrobials on germination and outgrowth of *C. perfringens* from spore inocula during continuous chilling is limited. Thippareddi et al. (2003) showed that buffered sodium citrate and buffered sodium citrate supplemented with sodium diacetate can be effectively used to inhibit *C. perfringens* outgrowth during chilling of roast beef and injected pork. Aran (2001) reported that sodium and potassium lactates can be used for inhibition of *C. perfringens* growth in processed beef goulash during isothermal storage conditions. The author reported that calcium lactate was more effective than calcium lactate and at either 1.5% or 3.0%, and that calcium lactate prevented outgrowth of *C. perfringens* even after 28 days of storage at 25 °C. The author attributed the improved antimicrobial activity, in part, to the ability of calcium lactate to lower the pH to 5.0 and 5.5 at 1.5% and 3.0% concentrations, respectively, from an initial pH of 6.0. However, in the present study, addition of sodium lactate slightly increased ($p \leq 0.05$) the pH of the injected turkey breast meat. It is possible that other ingredients in beef goulash and the injected turkey breast meat might have altered characteristics of the two products on addition of sodium and calcium lactates.

Meyer et al. (1995) reported similar inhibitory effects of sodium lactate and sodium diacetate on growth of nonpathogenic, nonproteolytic, psychrotrophic *Clostridium* species isolated from a spoiled (pink

discoloration and off-odor) cook-in-the-bag refrigerated turkey breast meat product during refrigerated storage. Reports by Maas et al. (1989), Meng (1992) and Miller et al. (1993) also indicate the antimicrobial effect on *C. botulinum* outgrowth and toxin production by sodium salts of organic acids.

The USDA-FSIS (USDA-FSIS, 1999) stabilization requirements for processed meat and poultry products require that the product be chilled at a rate sufficient to prevent multiplication of *C. botulinum* and restrict the germination and outgrowth to $<1.0 \log_{10}$ CFU/g increase in *C. perfringens*. It is evident that the germination and outgrowth potential for *C. perfringens* spores differs with each meat substrate and that it is dependent on other intrinsic characteristics, including the pH of the meat. Thus, care should be exercised when predictive models generated from model systems (broth or other meat types) are used to estimate *C. perfringens* growth during continuous chilling of meat products after thermal processing. Based on the results of the present study, chemical antimicrobials such as buffered sodium citrate alone or in combination with sodium diacetate or sodium salts of lactic or acetic acids can be used as ingredients in processed meat products to provide an additional measure of safety to address *C. perfringens* hazard during chilling of meat products.

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